



Expanding substrate scope of lipase-catalyzed transesterification by the utilization of liquid carbon dioxide



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ABSTRACT

Secondary alcohols having bulky substituents on both sides of the chiral center are often poor substrates for most lipases. Here we reported that substrate scopes of two of the most used lipases, *Candida antarctica* lipase B and *Burkholderia cepacia* lipase, were found to be expanded toward more bulky secondary alcohols such as 1-phenyl-1-dodecanol and 2-methyl-1-phenyl-1-propanol by simply using them in liquid carbon dioxide as a solvent. The effects of solvents, reaction pressure, and pre-treatment of the enzyme with liquid CO₂ on this acceleration phenomenon were also studied.

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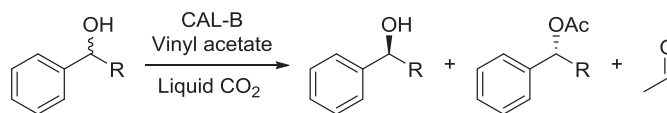
1. Introduction

Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3), have been well established as valuable catalysts for the preparation of enantiomerically-enriched compounds mainly related to pharmaceuticals and agrochemicals due to their broad substrate specificity, ease of use and high regio- and stereoselectivity. Among them, the lipase B from *Candida antarctica* immobilized on acrylic resin (Novozym 435, CAL-B) and *Burkholderia cepacia* lipase immobilized on ceramic carrier (Amano Lipase PS-C, BCL) have been by far among the most used biocatalysts due to their high stability in a variety of reaction media.¹ They retain most of their activities in nonaqueous media such as organic solvents, making them efficient to carry out esterification and transesterification reactions, in addition to the traditional hydrolysis reactions.

Especially, CAL-B has been effectively used in kinetic resolution of wide range of *sec*-alcohols with one large and one medium size substituents. The relationship between the structure of the substrates and the enantioselectivity has been shown to follow the empirical Kazlauskas's rule.² The alcohol-binding part of CAL-B contains a so-called 'stereoselective pocket' (defined by Thr42, Ser47 and Trp104), which gives the enzyme high substrate selectivity toward various *sec*-alcohols.³ However, this stereoselective pocket is only large enough to conformably accommodate an ethyl or smaller substituent under standard conditions.⁴ The lipase

shows decreased activity and selectivity when both substituents larger than an ethyl group are introduced. Due to this steric constraint the substrates having two bulky substituents cannot be accepted by the lipase under standard conditions.

We have recently reported on utilizing liquid carbon dioxide, which is emitted as waste through combustion of fossil fuels, as an efficient solvent for CAL-B catalyzed transesterification reaction (Scheme 1).⁵ The use of CO₂ as a solvent has a number of positive impacts on 'green chemistry', since it is an abundant, non-flammable, nontoxic, and chemically inert (under many conditions), environmentally acceptable source.^{6–8} Large-scale kinetic resolution of *rac*-1-phenylethanol using liquid CO₂ flow as a solvent in a continuous reactor was also successful and achieved a very low environmental factor.⁵ Additionally, it was found that the reactions of few substrates with both substituents slightly larger than ethyl proceeded but with very low yields. In this study, we found that CAL-B and BCL in liquid CO₂ can surprisingly expand their substrate scopes toward bulky secondary alcohols. The underlying mechanism for this liquid CO₂-induced effect was also studied.



Scheme 1. CAL-B catalyzed acetylation of alcohols in liquid CO₂.

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2. Results

2.1. Substrate specificity of CAL-B in liquid CO₂

The substrate specificity of CAL-B catalyzed kinetic resolution of a series of phenylalkanols was conducted systematically in liquid CO₂ and compared with those reactions in hexane. Hexane was chosen as the comparative solvent because CAL-B had already been proved to generally afford higher activity in hexane compared to other conventional solvents.⁵ Vinyl acetate was used as the acylating agent for an irreversible transesterification. For gaining impression on the accuracy, all reactions were performed in reactors with the same shape, and vigorously stirred with a magnetic bar to eliminate the mass transfer effects.

CAL-B showed high activity to 1-phenylethanol **1a** and 1-phenylpropanol **2a** with excellent enantioselectivity ($E > 200$) in liquid CO₂.⁵ When the methyl (**1a**) moiety was replaced by ethyl (**2a**) group, the reaction in liquid CO₂ became much faster than in hexane (Table 1).⁵ Therefore, more challenging substrates (**3–9a**),

which are normally difficult to react using conventional solvents, were examined in this report. Surprisingly, the enzyme exhibited activity to all bulky 1-phenylalkanols (**3–9a**) in liquid CO₂, whereas almost no reactivity was observed in hexane. Only with α -cyclopropylbenzyl alcohol (**8a**) the CAL-B showed a low activity in hexane (6% of conversion), while transformed up to 32% conversion with extremely high enantioselectivity ($E > 200$) in liquid CO₂. Moreover, the lipase afforded high conversions (ranging from 30% to 40%) regardless of the length of the alkyl substituents from propyl **3a** to dodecanyl **7a**, with an enantiomeric ratio (E) up to 32. This is the first report regarding successful resolution of the aforementioned bulky *sec*-alcohols having such long side chains by wild-type CAL-B.

2.2. Effect of acyl donor

There are several reports which stated that changing the acyl donor from vinyl acetate to other vinyl esters having longer alkyl chain, in some instances, could change enantioselectivity of the

Table 1
Substrate specificity of CAL-B catalyzed transesterification in hexane and in liquid CO₂

Substrate 1–9a	Solvent	Time (h)	Enzyme (mg)	Product 1–9b	<i>c</i> (%)	<i>ee</i> _p ^b (%)	<i>E</i>
1a 	Hexane Liquid CO ₂	1 ^a	10 ^a	1b 	31 ^a 32 ^a	>99 ^a >99 ^a	>200 ^a >200 ^a
2a 	Hexane Liquid CO ₂	18 ^a	10 ^a	2b 	4 ^a 25 ^a	N.d. ^a >99 ^a	N.d. ^a >200 ^a
3a 	Hexane Liquid CO ₂	120 (60 ^a)	100 (10 ^a)	3b 	4 (0 ^a) 38 (6 ^a)	N.d. 90	N.d. 32
4a 	Hexane Liquid CO ₂	120 (100 ^a)	100 (10 ^a)	4b 	2 (0 ^a) 30 (4 ^a)	N.d. 72	N.d. 8.0
5a 	Hexane Liquid CO ₂	120	100	5b 	2 40	N.d. 56	N.d. 5.1
6a 	Hexane Liquid CO ₂	120	100	6b 	<1 37	N.d. 66	N.d. 7.1
7a 	Hexane Liquid CO ₂	120	100	7b 	<1 30	N.d. 66 ^c	N.d. 6.4
8a 	Hexane Liquid CO ₂	16	20	8b 	6 32	N.d. 98	N.d. >200
9a 	Hexane Liquid CO ₂	120	100	9b 	<1 10	N.d. 98	N.d. 127

Reaction conditions: alcohols (**1–2a**: 0.4 mmol; **3–9a**: 0.2 mmol) with vinyl acetate (1.1 mmol) in 10 mL hexane or liquid CO₂ (7.0 MPa) at 20 °C. N.d.: not determined due to low conversions observed.

^a Data taken from reference, conditions were slightly different.⁵

^b *ee* of product.

^c Determined by chiral GC analysis of the resultant alcohol after hydrolyzing the corresponding ester by 4 M NaOH in MeOH.

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